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**(54) [Title of the Invention]**

A plant blight controlling agent which contains natural components as effective ingredients

**(57) [Abstract]**

[Objective] In the cultivation of plants in the areas of agriculture, horticulture and landscape gardening including golf courses, etc, the objective is to provide a plant blight controlling agent which is applied to the plants or to the soil for the purposes of preventing or eradicating plant blight (plant disease) originating in moulds and bacteria, etc, where said plant blight controlling agent is derived from natural materials consumed by humans as foods over a prolonged period and so are confirmed as being safe.

[Constitution] The plant blight controlling agent is composed of one or more essential oils or the main components of essential oils extracted from natural materials such as cinnamon, cassia, clove, hinoki (white cedar), eucalyptus, peppermint, spearmint, cumin, star anise and caraway which contain cinnamaldehyde, eugenol, hinokitiol, cineol, menthol, carvone, cuminal and anethole. Furthermore, it may contain one or more tea extracts or tea-derived components such as tea catechins or saponins obtained from tea by extraction and separation, or it may be a mixture of the aforesaid essential oils or main components thereof and the aforesaid tea extracts or tea-derived components.

**[Scope of Claims]**

**[Claim 1]** A plant blight controlling agent employing, as effective ingredients, natural components containing one or more extracts from natural flavours or the chief components thereof.

**[Claim 2]** A plant blight controlling agent employing, as effective ingredients, natural components containing one or more extracts or the undermentioned chief components from natural materials such as cinnamon, cassia, clove, hinoki, eucalyptus, peppermint, spearmint, cumin, star anise and caraway which contain cinnamaldehyde, eugenol, hinokitiol, cineol, menthol, carvone, cuminal or anethole.

**[Claim 3]** A plant blight controlling agent employing, as effective ingredients, natural components containing one or more from amongst cinnamaldehyde, eugenol, hinokitiol, cineol, menthol, carvone, cuminal and anethole, which are components extracted from natural materials.

**[Claim 4]** A plant blight controlling agent employing, as effective ingredients, natural components containing one or more extracts from tea or tea-derived components such as the tea catechins or tea saponins which are obtained by extraction and separation from tea.

**[Claim 5]** A plant blight controlling agent employing, as effective ingredients, natural components containing tea saponins obtained by extraction and separation from tea.

**[Claim 6]** A plant blight controlling agent employing natural components as effective ingredients, characterized in that it comprises a mixture of effective ingredients described in Claims 1, 2 or 3 and effective ingredients described in Claims 4 or 5.

**[Detailed Description of the Invention]**

[0001]

[Industrial Field of Application] The present invention relates to a plant blight controlling agent employed for preventing or eradicating plant blight caused by moulds or bacteria, etc, in the cultivation of plants in the areas of agriculture, horticulture and landscape gardening including golf courses, etc.

[0002]

[Prior Art] In the cultivation of plants in the areas of agriculture, horticulture and landscape gardening including golf courses, etc, chemically synthesized organo-phosphorous and organo-sulphur germicides have been used hitherto for the prevention and elimination of plant blight.

[0003]

[Problem to be Resolved by the Invention] However, while these germicides have an outstanding antimicrobial effect they are usually highly toxic, and there are potential adverse effects on humans and on fish due to breakdown of the soil ecosystem and leakage into the rivers and underground water, so demand is increasing for chemicals which are more highly safe.

[0004] Against this background, the present inventors have directed their attention to the safety of natural materials which are consumed by humans as foods over a long period and are thereby confirmed as being safe, and having taken note of the fact that amongst certain natural materials there has already in the past been confirmed an antimicrobial effect against toxins in foods such as botulinus, staphylococci and intestinal vibrio, etc, they have set out to develop a plant blight controlling agent derived from natural materials which has a preventive or eradicating effect against plant blight and, being a natural material, is also safe.

[0005]

[Means for Resolving the Problem] In order to resolve this problem, the present invention provides a plant blight controlling agent in which the effective ingredients are extracts or components from natural flavours, and extracts or components from tea.

[0006] Specifically, the present invention is a plant blight controlling agent employing, as effective ingredients, natural components containing one or more natural flavour extracts or chief components thereof. Those materials which have the most marked plant blight controlling (i.e. antimicrobial) effects as an active ingredient contain cinnamaldehyde, eugenol, hinokitiol, cineol, menthol, carvone, cuminal and/or anethole as their chief components, and it is extremely effective to produce the plant blight controlling agent with one or more of these chief components.

[0007] Furthermore, essential oils such as cinnamon oil, cassia oil, clove oil, hinoki oil, peppermint oil, eucalyptus oil, spearmint oil, cumin oil, star anise oil and caraway oil which are extracted from natural materials such as cinnamon, cassia, clove, hinoki, eucalyptus, peppermint, spearmint, cumin, star anise and caraway containing the above components also show a clear plant blight controlling action, and forming a plant blight controlling agent containing one or more of these essential oils is also preferred. It is of course possible to combine one or more of the aforesaid chief components and the aforesaid essential oils.

[0008] In addition, extracts of tea (including tea seeds; so too elsewhere below) or tea-derived components such as the tea catechins and tea saponins, in particular the tea saponins, which are obtained by extraction and separation from tea, also display effective action as plant blight controlling agents in which the effective ingredients are natural components, and it is possible to produce plant blight controlling agents containing one or more of these.

[0009] It is possible to enhance the plant blight controlling action still further by mixing together the aforesaid natural extracts, chief components thereof or essential oils, along with the tea extracts or tea-derived components such as tea saponins.

[0010] Now, explaining in more detail the plant blight controlling agent relating to the present invention, the natural materials which are the source of the extracts or components which constitute the effective ingredients of the plant blight controlling agent relating to the

present invention are those containing cinnamaldehyde, eugenol, hinokitiol, cineol, menthol, carvone, cuminal or anethole as a component.

[0011] For example, cinnamon and cassia contain cinnamaldehyde, cloves contain eugenol, hinoki contains hinokitiol, eucalyptus contains cineol, peppermint contains menthol, spearmint and caraway contain carvone, cumin contains cuminal, and star anise or anise contain anethole.

[0012] The extracts obtained by extraction of the fresh or dried leaves, stems, fruit or flowers, etc, of these natural materials with hot water or an organic solvent such as ethanol, methanol, acetone or ether, or the distillate obtained by steam distillation thereof, or again the cinnamaldehyde, eugenol, hinokitiol, cineol, menthol, carvone, cuminal and anethole separated and purified by the usual methods from the oil components obtained by distillation, can all be used as effective components of this plant blight controlling agent.

[0013] The tea employed in the extraction, or in the extraction and separation, of effective components for the plant blight controlling agent relating to the present invention is not particularly restricted providing it falls into the category of teas, and there can be used green tea or other such unfermented tea, Oolong tea or other such semi-fermented tea, black tea or other such fermented tea, or the unprocessed tealeaves of these. Here, an extract from tea means an extract obtained from such teas using hot water or a hydrophilic organic solvent such as ethanol, methanol or acetone, etc.

[0014] Reference to tea catechins means epigallocatechin, epicatechin gallate, epigallocatechin gallate and crude materials containing these catechins, and it also includes the theaflavins which are the enzymic oxides of the catechins contained in fermented tea and the enzymic oxides of the catechins of semi-fermented tea. Crude catechin from tea may be obtained by extracting tealeaves with hot water or a hydrophilic organic solvent such as ethanol or acetone, then performing a treatment to remove caffeine with chloroform, after which partitioning is carried out with ethyl acetate, followed by concentration. Moreover, if this is further separated by HPLC, the respective catechin purified products are obtained.

[0015] Tea saponins refers to the various saponins in tealeaves, the various saponins in tea seeds and the crude materials containing various saponins obtained from tealeaves and tea seeds. Purified tealeaf saponins are obtained by extracting tealeaves with methanol or water, further extracting with butanol and performing separation by column chromatography, while purified tea seed saponins are obtained by extracting defatted tea seeds with alcohol, dissolving the product in ether and hydrochloric acid and precipitating.

[0016] The methods for extracting and separating tea catechins and tea saponins described above are merely examples of the methods for obtaining the purified materials, and the extraction and purification of the tea components used as effective components in the plant blight controlling agent are not to be restricted to these particular methods.

[0017] The plant blight microorganisms against which the plant blight controlling agent of the present invention can be used include Rhizoctnia, Pythium, Curvularia, Helmintosporium and Pyricularia, but are not limited thereto. Furthermore, the plants which are subjected to blight prevention and control include grass, rice and the like, but the plant blight controlling agent can also be used for vegetables and fruit, etc.

[0018] The component composition of the plant blight controlling agent of the present invention and the amount applied will vary according to factors such as the particular disease microorganism which is the subject of prevention and control, the type of plant, the administration time and the environmental conditions, etc, so is not restricted. With regard to the form of use, the preparation employed can have the form or a liquid, gel or solid, etc, and the form is not particularly restricted. It can also be used adsorbed to a polymer absorbent or to a mineral such as zeolite.

[0019]

[Effects of the Invention] In accordance with the present invention, cinnamaldehyde, eugenol, hinokitiol, cineol, menthol, carvone, cuminal and anethole which are components extracted from natural materials, or extracts from flavours such as the essential oils extracted from cinnamon, cassia, clove, eucalyptus, peppermint, spearmint, cumin, star anise and caraway, etc, have an outstanding antimicrobial effect against plant blight microorganisms. Furthermore, tea extracts or tea components also have an antimicrobial effect.

Additionally, mixed compositions of these are shown to have a marked antimicrobial effect. Moreover, since these effective components are components derived from natural materials which can be used as foods and drinks by humans over a long time, there can be provided a plant blight controlling agent which is suitable for the prevention and control of plant blight and which has few adverse effects of the environment and is safe for humans.

[0020]

[Examples] Below the antimicrobial effects of the plant blight controlling agent relating to the present invention are explained in more specific terms by providing examples but, insofar as the essence of the invention is retained, there is to be no restriction to the examples given.

[0021] [Example 1] An investigation was performed into the growth inhibition effects against plant disease microorganisms of natural flavour extracts and components. Thus, a study was carried out into the effects of extracts of cinnamon, cassia, clove, eucalyptus, peppermint, spearmint, cumin, star anise, caraway and hinoki, and of cinnamaldehyde, eugenol, menthol and anethole which are components obtained from the cinnamon extract, clove extract, peppermint extract and star anise extract.

[0022] Dried bark of cinnamon, dried leaves of cassia, dried buds of clove, fresh leaves of eucalyptus, dried leaves of peppermint, dried whole spearmint, cumin fruit, dried fruit of star anise, fruit of caraway and dried

hinoki were respectively subjected to steam distillation and the cinnamon oil, cassia oil, clove oil, peppermint oil, eucalyptus oil, spearmint oil, cumin oil, star anise oil, caraway oil and hinoki oil obtained were used as the extracts from these natural materials.

[0023] Twice the amount of acidic sodium sulphite was added to the cinnamon oil, then dilute ethanol added and boiling performed, followed by cooling, filtering, washing with 80% ethanol and drying, after which the same quantity of sulphuric acid was added and the product obtained used as the cinnamaldehyde. Clove oil was dissolved in three times the amount of 10% sodium hydroxide solution, then leaching performed with ether and the terpenes removed, after which dilute sulphuric acid was added to the alkali solution, then distillation carried out under reduced pressure and the product obtained used as the eugenol. Peppermint oil was cooled and subjected to centrifuging, and the product obtained used as the menthol. Furthermore, the star anise oil was cooled and crystallized, and the product used as the anethole.

[0024] The investigation of the antimicrobial effects was carried out against three plant blight microorganisms, namely Rhizoctonia solani Kuhn (referred to below as microorganism R), Pythium aphanidermatum (referred to below as microorganism P) and Curvularia spp. (referred to below as microorganism C) which were respectively the cause of the "brown patch disease", "scorching disease" and "leaf wilt disease" which occur in the cold region type turf grasses used for golf courses and the like.

[0025] The investigation of the effects of these extracts and components in inhibiting the growth of the grass disease microorganisms was carried out by the agar dilution method using a potato-sucrose medium, in groups of three based on three concentration levels (50, 500 and 5000 ppm) for each microorganism. For each concentration, the percentage inhibition of growth was determined from the extent of development of the hyphae of the disease microorganisms compared to a group where no addition was made. A percentage inhibition of 100% indicates that there was zero development (elongation) of the hyphae, and a percentage inhibition of 50% indicates that there was 1/2 the development (elongation) of the hyphae. Now, in the case of microorganism P, investigation was carried out after culturing for 24 hours at 30°C, and in the case of microorganisms R and C it was after culturing for 3 days or 6 days at 25°C respectively. The aforesaid extracts and essential oil components were emulsified using a sucrose fatty acid ester and used in the test after dilution to the specified concentration.

[0026] The growth inhibition effects against microorganisms R, P and C are shown in Tables 1, 2 and 3 respectively. From the results shown, it is clear that the natural material extracts and components tested have an outstanding antimicrobial effect in that there was complete inhibition of each of the microorganisms at 500 ppm or 5,000 ppm.

[0027]

[Table 1]

Percentage Inhibition of Growth of Microorganism R by the Specified Natural Material Extracts and Components

| Test Material | Concentration | | |
|---|---|---|---|
| | 50 | 500 | 500 |
| (Extract) | | | |
| cinnamon oil | 64 | 100 | 100 |
| cassia oil | 51 | 100 | 100 |
| clove oil | 54 | 100 | 100 |
| hinoki oil | 92 | 100 | 100 |
| peppermint oil | 0 | 55 | 100 |
| eucalyptus oil | 0 | 50 | 100 |
| spearmint oil | 0 | 37 | 100 |
| cumin oil | 0 | 57 | 100 |
| star anise oil | 0 | 75 | 100 |
| caraway oil | 0 | 45 | 100 |
| (Component) | | | |
| cinnamaldehyde | 85 | 100 | 100 |
| eugenol | 57 | 100 | 100 |
| menthol | 47 | 100 | 100 |
| anethole | 10 | 100 | 100 |

[0028]

[Table 2]

Percentage Inhibition of Growth of Microorganism P by the Specified Natural Material Extracts and Components

| Test Material | Concentration | | |
|---|---|---|---|
| | 50 | 500 | 500 |
| (Extract) | | | |
| cinnamon oil | 9 | 100 | 100 |
| cassia oil | 23 | 100 | 100 |
| clove oil | 0 | 100 | 100 |
| hinoki oil | 100 | 100 | 100 |
| peppermint oil | 0 | 60 | 100 |
| eucalyptus oil | 0 | 63 | 100 |
| spearmint oil | 17 | 69 | 100 |
| cumin oil | 0 | 40 | 100 |
| star anise oil | 0 | 43 | 100 |
| caraway oil | 0 | 57 | 100 |
| (Component) | | | |
| cinnamaldehyde | 19 | 100 | 100 |
| eugenol | 23 | 100 | 100 |
| menthol | 0 | 100 | 100 |
| anethole | 4 | 100 | 100 |

[0029]

[Table 3

Percentage Inhibition of Growth of Microorganism C by the Specified Natural Material Extracts and Components

| Test Material | Concentration | | |
|---|---|---|---|
| | 50 | 500 | 500 |
| (Extract) | | | |
| cinnamon oil | 52 | 77 | 100 |
| cassia oil | 0 | 52 | 100 |
| clove oil | 35 | 79 | 100 |
| hinoki oil | 100 | 100 | 100 |
| peppermint oil | 0 | 51 | 100 |
| eucalyptus oil | 0 | 40 | 100 |
| spearmint oil | 0 | 48 | 100 |
| cumin oil | 0 | 63 | 100 |
| star anise oil | 0 | 38 | 100 |
| caraway oil | 0 | 49 | 100 |
| (Component) | | | |
| cinnamaldehyde | 19 | 100 | 100 |
| eugenol | 23 | 100 | 100 |
| menthol | 0 | 100 | 100 |
| anethole | 0 | 100 | 100 |

[0030] [Example 2] An investigation was made into the growth inhibition effects of tea extracts and tea components against the plant disease microorganisms. The investigation of the growth inhibition effects was carried out with green tea, Oolong tea and black tea extracts and using tea components comprising tea crude catechin and tea seed saponin.

[0031] As the green tea, Oolong tea and black tea extracts, there were used extracts obtained by extracting the respective tealeaves for 10 minutes with 20 times the amount of hot water, and then freeze-drying under vacuum. Tea crude catechin (comprising about 70%

of the total catechin) was obtained by extracting green tea with ten times the amount of hot water, then concentrating under reduced pressure, followed by extracting with distilled water and 15% acetone solution on a chromatography column, after which further concentration and freeze drying were performed. In the case of the tea seed crude saponin, the tea seeds were defatted and then extracted with alcohol.

[0032] The plant disease microorganisms used were the same three types of turfgrass disease microorganisms as in Example 1. Furthermore, the detection of the growth inhibition effect was carried out based on the method described in Example 1.

[0033] The percentage inhibition of the growth of microorganism R by the test materials is shown in Table 4, and similarly Table 5 and Table 6 show the results in the case of microorganisms P and C respectively. From these results, it can be seem that all the test materials exhibit an inhibitory effect on the growth of the disease microorganisms, and it is clear that the percentage inhibition increases as the employed concentration is increased.

[0034]

[Table 4]

Percentage Inhibition of the Growth of Microorganism R by Tea Extracts and Tea Components

| Test Material | Concentration | | |
|---|---|---|---|
| | 50 | 500 | 500 |
| green tea hot water extract | 0 | 3 | 60 |
| Oolong tea hot water extract | 0 | 31 | 85 |

| | | | |
|---|---|---|---|
| black tea hot water extract | 0 | 3 | 81 |
| tea crude catechin | 0 | 3 | 89 |
| tea seed crude saponin | 37 | 67 | 75 |

[0035]

[Table 5]

Percentage Inhibition of the Growth of Microorganism P by Tea Extracts and Tea Components

| Test Material | Concentration | | |
|---|---|---|---|
| | 50 | 500 | 500 |
| green tea hot water extract | 0 | 20 | 88 |
| Oolong tea hot water extract | 0 | 13 | 100 |
| black tea hot water extract | 0 | 25 | 100 |
| tea crude catechin | 0 | 19 | 99 |
| tea seed crude saponin | 0 | 5 | 65 |

[0036]

[Table 6]

Percentage Inhibition of the Growth of Microorganism C by Tea Extracts and Tea Components

| Test Material | Concentration | | |
|---|---|---|---|
| | 50 | 500 | 500 |
| green tea hot water extract | 0 | 3 | 43 |
| Oolong tea hot water extract | 0 | 0 | 41 |
| black tea hot water extract | 0 | 5 | 43 |
| tea crude catechin | 3 | 15 | 72 |
| tea seed crude saponin | 19 | 69 | 89 |

[0037] [Example 3] An investigation was carried out to determine if there was a synergistic effect in terms of the inhibition of the growth of microorganisms R and P using a mixture of the aforesaid specified natural material extracts and tea extracts. A total of 17 compositions were prepared by varying the contents of

five components, namely the Oolong tea hot water extract, cinnamon oil, clove oil, peppermint oil and hinoki oil used in Examples 1 and 2 (Oolong tea extract 20-50%, cinnamon oil 20-40%, peppermint oil 10-40%, hinoki oil 20-40%), and the growth inhibition effects against the turfgrass disease microorganisms P and R used in Examples 1 and 2 determined. The determination of the effects was carried out in groups of three based on three concentration levels using the agar dilution method, employing a potato-sucrose medium. The percentage inhibition of the growth of the disease microorganisms at each concentration was plotted as a logarithmic graph and the 50% inhibitory concentration $ED_{50}$ (median effective dose) determined. By comparison with the $ED_{50}$ value for the respective essential oils alone, the synergistic effect of the Oolong tea extract was determined. The culturing conditions of the disease microorganisms were the same as in Example 1.

[0038] Amongst the 17 compositions, those where a synergistic effect by the Oolong tea was seen are shown in Table 7, together with the results thereof.

[0039]

[Table 7]

Growth Inhibition Effect of the Individual Materials and Mixed Compositions on Microorganisms R and P (concentration in ppm at 50% growth inhibition)

| Test Material (composition) | $ED_{50}$ against Microorganism R | $ED_{50}$ against Microorganism P |
|---|---|---|
| Oolong tea extract 100% | 1136 | 1333 |
| cinnamon oil 100% | 36 | 63 |
| clove oil 100% | 47 | 108 |
| peppermint oil 100% | 425 | 355 |

| | | |
|---|---|---|
| hinoki oil 100% | 26 | 33 |
| mixed composition A | 19 | 55 |
| mixed composition B | 27 | 26 |

[0040] Aforesaid mixed composition A comprised 20% Oolong tea extract, 40% cinnamon oil and 40% clove oil, and had the most marked inhibitory effect against microorganism R. Aforesaid mixed composition B comprised 20% Oolong tea extract, 10% cinnamon oil, 30% peppermint oil and 40% hinoki oil, and had the most marked inhibitory effect against microorganism P.

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